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Biological relevance of polyploidy: ecology to genomics
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Genetic and epigenetic reprogramming of the wheat genome upon allopolyploidization

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In the past few years we have analysed alterations in genome structure and expression that occur in wheat upon allopolyploidization. Our major findings in natural and synthetic allopolyploid wheat are reviewed here. It was found that allopolyploidization brings about rapid genome evolution through the instantaneous generation of a variety of cardinal genetic and epigenetic alterations comprising: (1) non-random elimination of coding and non-coding DNA sequences, (2) epigenetic changes such as DNA methylation of coding and non-coding DNA leading, among others, to gene silencing, and (3) activation of retroelements, which in turn alters the expression of adjacent genes. These changes were reproducible, occurring in the F1 hybrids or in the first generation(s) of a series of nascent allopolyploids corresponding to various interspecific and intergeneric combinations. Moreover, these changes were similar to those that occurred twice in nature: first, at the transition from diploid to tetraploid wheat (~0.5 Mya) and, second, at the transition from tetraploid to hexaploid wheat (~9500 years ago). Elimination of non-coding sequences augments the differentiation of homoeologous chromosomes at the polyploid level, thus increasing the physical divergence between homoeologues and contributing to the diploid-like meiotic behaviour of polyploid wheat. Transcriptional and post-transcriptional alterations of gene activity, including transcriptional activation of retroelements, led to novel expression patterns. These phenomena emphasize the plasticity of the genome with regard to both structure and gene expression. This plasticity in turn might improve the adaptability of the newly formed allopolyploids and facilitate their rapid and successful establishment in nature. *Biological Journal of the Linnean Society*, 2004, **82**, 607–613.

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INTRODUCTION

In recent years there has been a renewed interest in polyploidy and several works have analysed the role of polyploidy in speciation and its impact on genome evolution and gene expression. The major advances in this field are described in this special issue of the journal. Here, we focus on the genetic and epigenetic variation released at the very early stages of allopolyploidization in wheat. The wheat group (the genera *Aegilops* and *Triticum*) constitutes an allopolyploid series containing diploid, tetraploid and hexaploid species (Feldman, Lupton & Miller, 1995). Durum (macaroni) wheat, *T. turgidum* ssp. *durum*, contains two diploid genomes ($2n = 4x = 28$; genome BBAA) and bread wheat, *T. aestivum* ssp. *aestivum*, contains three diploid genomes ($2n = 6x = 42$; genome BBAADD). Their cytogenetic structure and origin have been intensively investigated over the last eight decades because of the obvious importance of these crops for human nutrition. Consequently, they have served as a classical model for evolution through allopolyploidy. The A and D genomes share a high degree of homology with the diploid genomes of *T. urartu* ($2n = 2x = 14$; genome AA) and *Ae. tauschii* ($2n = 2x = 14$; genome DD), respectively. The progenitor of the B genome, which contributed the cytoplasm

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of durum and bread wheat, remains uncertain because a wild species with a high degree of homology to the B genome of wheat has not been found. The diploid wheat most closely related to the B genome is *Ae. speltoides* ($2n = 2x = 14$; genome SS). The elusive nature of the B genome can be explained by one of the following possibilities: the diploid progenitor still exists but has not yet been discovered; it is extinct; or the B genome of wheat has rapidly evolved in the polyploid condition through genetic rearrangements, and introgression of chromosomal segments from other allopolyploids or diploids. Recent attempts at determining the timing of speciation events in the wheat group, based on molecular data, suggest that the diploid progenitors of wheat have diverged from a common progenitor 2.5–4.5 Mya (Huang *et al*., 2002). This divergence is relatively recent; therefore, we do expect a high degree of synteny and homology in DNA sequences from the three genomes. To a great extent, this is indeed the case: in hexaploid wheat, homoeologous chromosomes can compensate for each other's absence (Sears, 1954) and most genes or RFLP markers hybridize to three homoeologous sequences, i.e. are triplicated in hexaploid wheat (Gale *et al*., 1995; McIntosh *et al*., 1998). However, some sequences are found in only one genome or chromosome of hexaploid wheat (Fig. 1). Given the evolutionary history of wheat, the origin of genome-specific (GSS) or chromosome-specific (CSS) sequences is particularly intriguing and has been the original object of our research.

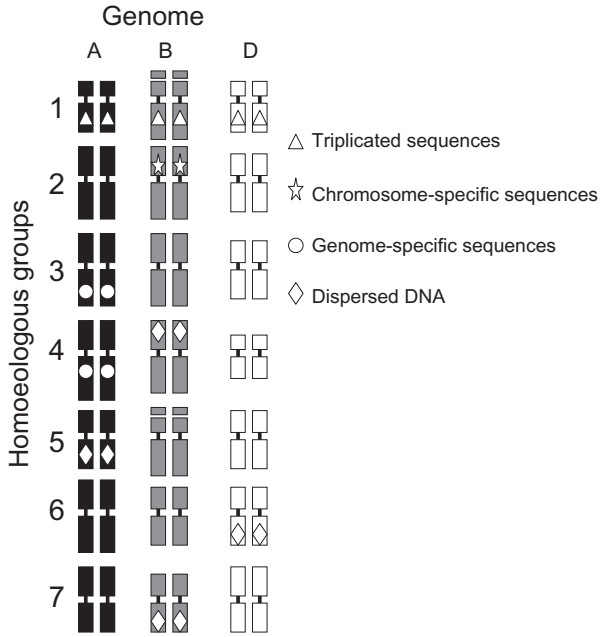

Figure 1. Schematic representation of the wheat karyotype. The wheat karyotype is arranged into genomes A, B and D and into seven homoeologous groups. This arrangement is after Sears (1954), who classified homoeologous chromosomes based on their ability to compensate for each other's absence. Examples of the different types of sequences are drawn on top of the chromosomes, namely: triplicated sequences, chromosome-specific sequences (CSSs) that are present in only one chromosome pair, genome-specific sequences (GSSs) that can be on more than one chromosome pair but only in one of the genomes, and dispersed repeats that are present on both homoeologous and non-homoeologous chromosomes.

THE ORIGIN OF GSS AND CSS SEQUENCES

Sequences such as GSSs or CSSs, which do not have a homoeologous counterpart, have either appeared in one genome after divergence from the common progenitor, as a result of accelerated evolution or of horizontal gene transfer, or they are present in all diploid progenitors and were lost in the allopolyploid background. Following the isolation of genome and chromosome-specific sequences from a library of DNA obtained through microdissection of the long arm of chromosome 5B (Vega *et al*., 1994), we initiated a series of studies aimed at understanding the origin of these sequences. We showed that the diploid relatives of wheat all contained sequences homologous to sequences that are present in only one genome or chromosome of tetraploid or hexaploid wheat (Feldman *et al*., 1997; Liu *et al*., 1997). This indicated that these sequences were lost in the course of wheat evolution. An intriguing observation was that the same sequences were lost twice: once upon tetraploid formation, ~0.5 Mya (Huang *et al*., 2002) and a second time, only ~9500 years ago (Feldman, 2001) as shown in Figure 2. These data led us to formulate a working hypothesis, namely that both GSSs and CSSs are rapidly eliminated from one genome after allopolyploidization, and that elimination might be reproducible, as suggested by the repeated elimination of the same sequences in both tetraploid and hexaploid wheat. In support of this hypothesis, the analysis of a synthetic hexaploid wheat, *T. turgidum* ssp. *dicoccoides* – *Ae. tauschii* (genome BBAADD) showed that sequences present in both parental accessions were lost from one genome of the amphiploid and thus had become GSSs and CSSs (Feldman *et al*., 1997; Liu *et al*., 1998). This suggested that DNA elimination had occurred immediately upon allopolyploidization. The synthetic hexaploid material that was analysed had been propagated for a number of generations and the precise parental plants used to synthesize the amphiploid were not available. Therefore, the production of a series of amphiploids derived from known homozygous plants was initiated (Ozkan, Levy & Feldman, 2001). These amphiploids, representing 22 different genomic combinations, were designed in order to address the

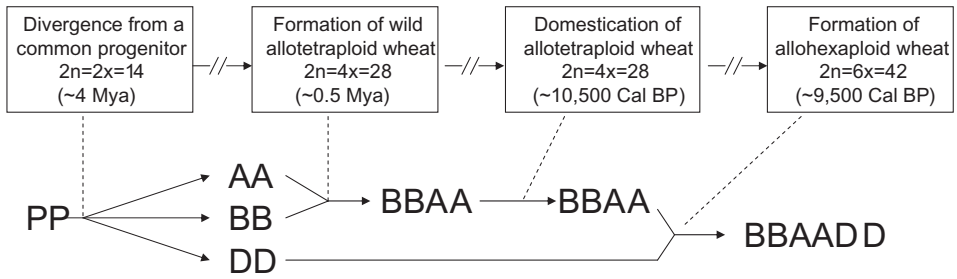

Figure 2. Evolutionary history of allotetraploid and allohexaploid wheat. Diploid wheats ($2n = 2x = 14$), from the *Triticum–Aegilops* group have diverged ~4 Mya from a common diploid progenitor (Huang *et al*., 2002), whose genome is indicated here as PP. Intergeneric hybridization between the diploid *T. urartu* (genome AA) as male and the donor of the B genome (an unknown species similar to *Ae. speltoides*) as female, followed by chromosome doubling, gave rise (~ 0.5 Mya) to the wild allotetraploid wheat, *Triticum turgidum,* ssp. *dicoccoides* ($2n = 4x = 28$, genome BBAA). This is the direct progenitor of durum and bread wheat. Domestication of allotetraploid wheat took place ~10 500 years ago (adapted from Feldman, 2001; Salamini *et al*., 2002) and was rapidly followed (~9500 years ago) by a second round of intergeneric hybridization and chromosome doubling between domesticated allotetraploid wheat and the donor of the D genome, *Ae. tauschii* ($2n = 2x = 14$, genome DD), giving rise to bread wheat, an allohexaploid with $2n = 6x = 42$ chromosomes (genome BBAADD).

time course of sequence elimination, its reproducibility, the various types of intergenomic interactions and the possible role of the cytoplasm. It was found that the GSSs and CSSs that existed in all the parental plants were rapidly eliminated from one genome of the synthetic allopolyploids. Elimination of GSSs was already initiated in F_1 plants and was completed in the second or third allopolyploid generation, whereas elimination of CSSs started in the first allopolyploid generation and was completed in the second or third generation. Sequence elimination started earlier in allopolyploids whose genomic combination was analogous to natural polyploids compared with allopolyploids that do not occur in nature. Elimination was a non-random and reproducible event whose direction was determined by the genomic combination of the allopolyploid. It was not affected by the genotype of the parental plants, by their cytoplasm or by the ploidy level and it did not result from intergenomic recombination (Ozkan *et al*., 2001).

In summary, the series of works described above showed that CSSs and GSSs that underwent elimination during wheat evolution, i.e. under natural conditions, were also eliminated in the synthetic amphiploids. This, together with the finding of sequence elimination in cases of spontaneous chromosome doubling (Ozkan *et al*., 2001), suggests that the experimental system used here, including the colchicine treatment, did not affect the elimination process. Sequence elimination in synthetic amphiploids, leading to the formation of CSSs and GSSs, might be a faithful repetition of the events that occurred in nature at the very early stages of allopolyploid wheat formation.

THE EXTENT OF ALLOPOLYPLOIDY-INDUCED DNA AND GENE LOSS

The works by Feldman *et al*. (1997), Liu *et al*. (1997, 1998) and Ozkan *et al*. (2001) dealt with GSS and CSS probes, i.e. a preselected set of loci that underwent elimination from one genome in allopolyploid wheat immediately after allopolyploidization. In an additional study we attempted to assess the extent of sequence elimination as well as to test whether other types of DNA rearrangements also occurred in a set of random, unselected loci. To do that, we carried a DNA fingerprinting of the diploid parents and compared it with that of the derived F_1 and amphiploids (Shaked *et al*., 2001). In total, 3661 loci were analysed by the amplified fragment length polymorphism (AFLP) method (Vos *et al*., 1995). However, the percentage of bands that disappeared could be estimated only from the polymorphic bands because of the dominant nature of AFLP. Our data (Shaked *et al*., 2001) indicated that between 5 and 14% of the loci could be eliminated (depending on the interspecific hybrid or the amphiploid studied). Moreover, sequence elimination was the prominent type of rearrangement, and the type of sequences affected, as determined by sequence analysis and Southern blotting, were mostly low-copy, non-coding DNA sequences. Interestingly, these numbers are in the same range as the 4–8% reduction in genome size of the amphiploids as determined by measurements of *C*-values in the diploid parents and in their allopolyploid derivatives (Ozkan, Tuna & Arumugnathan, 2003). They are also consistent with the lower than expected genome size of amphiploids in most angiosperms (Leitch & Bennett, 2004 – this

issue). This suggests that DNA elimination following polyploidy is a genome-wide event that may be widespread in plants. In a subsequent study (Kashkush, Feldman & Levy, 2002), we analysed 3072 transcripts using the cDNA–AFLP method (Bachem *et al*., 1996) in diploid parents and in the first generation of the derived amphiploid. We found that 60 of the 3072 loci were not additively inherited in the amphiploid. Forty-eight transcripts disappeared, partly because of gene loss and partly because of gene silencing as discussed below.

In summary, DNA elimination is a major response of the wheat genome to allopolyploidy. It affects mostly non-coding DNA but genes may also be lost during polyploidization.

THE EPIGENETIC IMPACT OF ALLOPOLYPLOIDY IN WHEAT: METHYLATION, SILENCING AND ACTIVATION

In addition to DNA rearrangements, epigenetic alterations were also studied in two synthetic allotetraploids. First, we analysed alterations in patterns of cytosine methylation throughout the genome (Shaked *et al*., 2001). This was done using methylation-sensitive amplification polymorphism (MSAP) (Xiong *et al*., 1999), a method derived from AFLP but using a pair of isoschisomer restriction enzymes, each with a different sensitivity to cytosine methylation. This analysis indicated that in 13% of the genomic DNA loci analysed, the parental patterns of methylation were altered in the allotetraploids, i.e. cytosine residues became either methylated or demethylated. Alterations in methylation affected both low-copy and repetitive DNA. Among the repetitive DNA sequences, retroelements were found to be affected by alterations in cytosine methylation.

When 3072 transcribed loci were analysed, using cDNA–AFLP (Kashkush *et al*., 2002), we observed new, non-additive patterns of gene expression in the allotetraploid, namely we found that 48 transcripts disappeared in the allotetraploid and 12 transcripts that were absent in the diploid parents appeared in the allotetraploid. These changes were reproducible in two independently made allotetraploids. Disappearance of the majority (~2/3) of the transcripts could be related to gene silencing rather than to gene loss. Northern analysis, together with RT-PCR, indicated that silencing of one or of both parental transcripts could occur. Silencing was partly (but not always) associated with cytosine methylation.

Among the 12 transcripts that were activated in the amphiploid, sequence homology was found only to retroelements. In this group, the WIS 2-1A element was further characterized. It was strongly activated in the amphiploid whereas a transcript was barely detectable in the diploid parents as determined by Northern blot analysis. WIS 2-1A RNA was reverse transcribed, giving rise to a putative linear cDNA transposition intermediate. However, when evidence for transposition were searched among ~1000 WIS 2-1A copies, no new insertions were found. This suggests that despite a strong transcriptional activation there are little if any new transposition events.

Long-terminal repeat (LTR) retroelements are known to have readout promoter activity and specific cases were reported of activation of genes by retroelements (Horowitz *et al*., 1984). Given the large copy number (tens of thousands) of WIS 2-1A elements in the genome, we hypothesized that transcriptional activation of WIS 2-1A elements could lead to both activation and silencing of adjacent genes. This hypothesis was tested by isolating chimeric transcripts that contained the retroelement termini fused to flanking sequences. Such transcripts were isolated using a method similar to transposon display, but with cDNA instead of genomic DNA (Kashkush, Feldman & Levy, 2003). We analysed in detail 22 such chimeric transcripts out of the 360 identified. These transcripts were altered in the amphiploid, i.e. were either present at the diploid level but disappeared in the amphiploid or were absent at the diploid level but appeared in the amphiploid. We showed that LTRs positioned in the 3′ end of genes did not affect the gene activity when silent but, when activated, these LTRs drove the transcription of antisense RNA and subsequent silencing of the adjacent genes. Conversely, when readout transcription from the LTRs was in the same orientation as the adjacent gene this caused an overexpression or misexpression of the adjacent gene. There was also production of new RNA species that had no homology to known genes and probably corresponded to non-coding genomic DNA adjacent to the LTR. These non-coding sequences are perhaps just 'junk' RNA or might have unknown regulatory functions.

In summary, retroelement transcriptional activation could have a genome-wide effect on adjacent genes, leading to activation or silencing of these genes.

DISCUSSION

In a recent review (Levy & Feldman, 2002), we have described the evolutionary impact of allopolyploidy as a two-phase process: first, the immediate response in the nascent amphiploid; and second, the changes, over long periods, that are facilitated by the allopolyploid condition, e.g. intergenomic transfer through recombination, transposition or introgression, translocations, allelic diversification, etc. (see summary in Table 1). Here we focused on the impact of allopolyploidization,

Table 1. Genetic and epigenetic alterations in wheat associated with allopolyploidy

A. Revolutionary changes (occurring immediately after allopolyploidization)
Structural level (genetic changes)
Elimination of DNA sequences from homoeologous chromosomes and genomes
Gene loss
Amplification or reduction of repetitive sequences
Chromosomal repatterning (translocations)
Elimination of rRNA genes (nucleolar organizers)
Chromatin remodelling due to methylation and acetylation
Gene expression level (epigenetic changes)
Gene inactivation or activation through alterations in cytosine methylation
Alteration of gene expression through transcriptional activation of retroelements:
Gene activation through readout from adjacent LTR-promoters
Gene silencing via antisense RNA through readout from adjacent LTRs
Intergenomic interactions (suppression, activation, dosage compensation)
B. Evolutionary changes (occurring during the life of the allopolyploid)
Structural level (genetic changes)
Intergenomic horizontal transfer of chromosomal segments
Introgression of chromosomal segments from other allopolyploids and formation of recombinant genomes
Gene inactivation through mutations, insertions and deletions
Functional diversification of homoeoalleles through mutations
Gene expression level (epigenetic changes)
Functional diversification of homoeoalleles through heritable epigenetic alterations

in the first generations of the allopolyploid's life, on genome structure as well as on gene expression. DNA elimination of both coding and non-coding sequences was a major, rapid and reproducible response of the genome to allopolyploidy. It is responsible for the origin of CSSs and GSSs in the genome of polyploid wheat. New patterns of gene expression were observed in the amphiploid. Gene silencing affected ~2% of the loci studied. This value is an underestimate because quantitative changes in transcript expression were not addressed and because monomorphic bands were not informative. Gene silencing was partly associated with cytosine methylation and partly with retroelement-mediated antisense silencing. Retroelements were strongly activated by amphiploidy and in turn affected the activity of adjacent genes. These data suggest that amphiploidy is a major genomic stress that leads to a genetic and epigenetic reprogramming of the genome. This reprogramming occurs early in the life of the nascent amphiploid, possibly in the zygote of the interspecific or intergeneric F_1 hybrid and/or right after chromosome doubling. The cascade of events leading to this reprogramming, the timing and tissues where it occurs and the underlying mechanisms are still unknown and remain a fascinating research topic.

The genome-wide genetic and epigenetic alterations triggered by allopolyploidy emphasize the great plasticity of the wheat genome, namely the ability of the resident genomes to alter their structure and expression when in the allopolyploid background. The reproducibility of the genetic and epigenetic events described here is indicative of a programmed rather than a chaotic response. This suggests that allopolyploidy is sensed in a specific way that activates a specific response rather than a random mutator response.

The impact of these events (gene elimination, silencing or activation) might be disruptive, neutral or increase fitness of the newly formed allopolyploid. It is hard to imagine that evolution would maintain such a sophisticated genomic response if it were disruptive or neutral. We discuss below the possible beneficial effects of the responses described here.

First, at the structural level, DNA elimination of 5–14% of the total genome increases the physical divergence between the diploid genomes that reside in the same allopolyploid nucleus. Such divergence can reduce the chances for homoeologous pairing, resulting in a diploid-like meiotic behaviour of the nascent allopolyploid that increases its fertility and maintains its disomic behaviour. The CSSs tend to cluster in several regions on each arm (Liu *et al*., 1997). These regions, which are the only homologous-specific regions, are assumed to play an important role in homology search and initiation of homologous pairing at meiotic prophase. At the gene expression level, all the events that involve genes (as summarized in Table 1), e.g. gene elimination or gene silencing, may also contribute to the rapid diploidization of the nascent amphiploid by eliminating or silencing redundant functions or by adjusting gene dosage to an opti-

mal level that suits the new polyploid condition and perhaps prevents a contradictory action by genes of the different genomes. We cannot give concrete examples that would exemplify how changes in gene expression would contribute to the organism's fitness, but mechanisms obviously exist that make adjustments at the expression level possible.

The panoply of epigenetic responses of the genome to polyploidy seems to be broad and sophisticated (Adams *et al*., 2003). For example, we have shown cases in which homoeologous transcripts from both diploid parents were silenced in the amphiploid. Antisense RNA might target the transcripts of both homoeoalleles and thus provide the underlying mechanism for this type of silencing. Retroelements are often considered as selfish DNA, which at best remain silent and at worse cause mutations. Our work highlights their role as controlling elements. We and others have shown that retroelements can activate adjacent genes (Horowitz *et al*., 1984) and thus can induce new patterns of gene expression. We show new evidence that they can also silence adjacent genes on a genome-wide scale thanks to their readout activity by *cis*-antisense RNA. They might also affect homoeoalleles or whole gene families in *trans* via antisense RNA. We estimated that a few hundred genes might be affected by this mechanism. This number is surprising as it raises many questions on how the genome deals with such a disruption in normal activity. A possible solution to this riddle is that when a new insertion near a gene has a disruptive effect, it is discarded by natural selection. According to such a hypothesis the tolerated insertions would be either neutral or beneficial. Deleterious ones would be recent insertions that have not yet been discarded by natural selection. This hypothesis is testable by determining the 'age' of retroelement-gene promiscuity. Additional mechanisms of gene expression regulation are probably active in wheat but have not yet been assessed in nascent allopolyploids nor on a genome-wide scale. For example, recent findings in natural and synthetic cotton allopolyploids show that silencing is not an 'on–off' phenomenon but that it can be differential, i.e. that one homoeoallele can be silenced in one tissue but expressed in the other (Adams *et al*., 2003; Adams & Wendel, 2004 – this issue). These findings suggest that homoeoalleles may 'specialize' or may even acquire new patterns of expression in an amphiploid background. With the exception of early works (Galili, Levy & Feldman, 1986), gene dosage response analysis using the modern tools of genomics have not been performed in wheat. Similarly, studies showing how the dosage of particular genes may increase fitness, as shown for the FLC locus in *Brassica* (Osborn *et al*., 2003), are still missing in wheat.

In summary, we are starting to discover the rapidity and the extent of re-programming of the genome, as a result of allopolyploidy in wheat. We are also starting to appreciate the complexity and the variety of mechanisms involved in the genetic and epigenetic response to polyploidy. Wheat and other species probably have both similar and different pathways to deal with genome duplication. For example, cotton, unlike wheat, shows genomic homoeostasis (Wendel, 2000) but is efficient at epigenetic reprogramming (Adams & Wendel, 2004). *Brassica* (Pires *et al*., 2004 – this issue) and *Arabidopsis* (Chen *et al*., 2004 – this issue) show random genetic rearrangements, unlike the programmed DNA elimination in wheat allopolyploids. The precise mechanisms whereby DNA elimination occurs, or the sensing and signalling leading to the epigenetic alterations and changes in gene expression are unknown and promise to be exciting fields of research using the wheat system. Last, but not least, a major challenge will be to understand if and how the various genetic and epigenetic alterations described here increase fitness of the nascent allopolyploid so that, equipped with the advantages of allopolyploidy (e.g. permanent heterosis between homoeoalleles), it can out-compete its two parents.

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